

Pervasive effects of *Wolbachia* on host activity

Michael T.J. Hague^{1,*}, H. Arthur Woods¹, and Brandon S. Cooper¹

¹Division of Biological Sciences
University of Montana
32 Campus Dr.
Missoula, MT 59812

***Corresponding Author**

Michael T.J. Hague
Division of Biological Sciences, University of Montana
32 Campus Dr. HS 104, Missoula, MT 59812
(406) 243-5122
michael.hague@mso.umt.edu

1 **ABSTRACT**

2 Heritable symbionts have diverse effects on the physiology, reproduction, and fitness of
3 their hosts. Maternally transmitted *Wolbachia* are one of the most common endosymbionts in
4 nature, infecting about half of all insect species. We test the hypothesis that *Wolbachia* alter host
5 behavior by assessing the effects of 14 different *Wolbachia* strains on the locomotor activity of
6 nine *Drosophila* host species. We find that *Wolbachia* alter the activity of six different host
7 genotypes, including all hosts in our assay infected with *w*Ri-like *Wolbachia* strains (*w*Ri, *w*Suz,
8 *w*Aur), which have rapidly spread among *Drosophila* species in only the last 13,000 years. While
9 *Wolbachia* effects on host activity were common, the direction of these effects varied
10 unpredictability and sometimes depended on host sex. We hypothesize that the prominent effects
11 of *w*Ri-like *Wolbachia* may be explained by patterns of *Wolbachia* titer and localization within
12 host somatic tissues, particularly in the central nervous system. Our findings support the view
13 that *Wolbachia* have wide-ranging effects on host behavior. The fitness consequences of these
14 behavioral modifications are important for understanding the evolution of host-symbiont
15 interactions, including how *Wolbachia* spread within host populations.

16 INTRODUCTION

17 Insects harbor microorganisms that have wide-ranging effects on their performance and
18 fitness [1–3], including manipulations to reproduction [4–7], provisioning of nutrients [1,8,9],
19 modifications of thermotolerance [10,11], and defense against pathogens [12–15]. Microbes may
20 also alter host behavior [16–21]. In extreme instances, parasitic microbes can induce behaviors
21 that increase the likelihood of transmission—for example, by directing hosts to habitats that
22 promote transmission [22–28]. Infected hosts may also change their own behavior as an immune
23 strategy against infection, including seeking warm temperatures to induce a “behavioral fever”
24 [29,30] or reducing activity and increasing sleep time [19,31–34]. Such behavioral modifications
25 have important implications for microbe spread and host fitness.

26 Maternally transmitted *Wolbachia* are the most common endosymbionts in nature,
27 infecting many arthropods [5,35,36] and two distantly related groups of nematodes [37].
28 Discordant *Wolbachia* and host phylogenies indicate that many hosts have recently acquired
29 *Wolbachia* via introgressive and horizontal transfer [38–43]. *Wolbachia* are primarily transmitted
30 vertically by female hosts, so natural selection favors beneficial effects on host fitness that
31 promote spread [44–47]. Maternal transmission occurs via the host reproductive system, but
32 *Wolbachia* are also found in host somatic tissues, including nervous, digestive, and metabolic
33 tissues [48–51]. Still, the behavioral and physiological consequences of somatic infections are
34 poorly understood [19,51].

35 Prior work indicates *Wolbachia* influence several host behaviors [19,52,53], including
36 sleep [54–56] and temperature preference [20,57,58]. We broadly test for *Wolbachia* effects on
37 the locomotor activity of *Drosophila* hosts infected with A-group *Wolbachia* ($N = 11$), B-group
38 *Wolbachia* ($N = 1$), and an A- and B-group co-infection ($N = 1$). Our analysis includes two
39 prominent A-group clades that recently spread among *Drosophila*: wMel-like *Wolbachia*
40 (wMelCS, two wMel variants, wYak, wSan, and wTei) and wRi-like *Wolbachia* (wRi, wSuz, and
41 wAur) [42,43]. We find that *Wolbachia* effects on host activity are common, particularly for
42 wRi-like *Wolbachia*, a “super-spreader” strain that rapidly spread among *Drosophila* species in
43 the last ~13,000 years [42].

44

45

46

47 **METHODS**

48 *Fly lines*

49 We evaluated 13 different *Wolbachia*-infected host genotypes (Figure 1, Table S1),
50 consisting of nine *Drosophila* species infected with 14 different A- and B-group *Wolbachia* that
51 diverged up to 46 million years ago [59]. For two of the host species, *D. melanogaster* and *D.*
52 *simulans*, we tested multiple *Wolbachia*-infected genotypes. This included a *D. simulans* host co-
53 infected with A-group wHa and B-group wNo [60–63]. We used tetracycline treatment as
54 previously described [20] to generate uninfected genotypes to pair with each infected genotype,
55 while taking care to avoid detrimental effects of the antibiotic treatment on mitochondrial
56 function [64] (see Supplemental Methods).

57

58 *Host locomotor activity assays*

59 We reared flies at 25°C under a 12-h light:12-h dark cycle (Percival model I-36LL) on a
60 standard food diet [20]. Each day, we collected a batch of female and male virgins for one pair of
61 uninfected and infected genotypes. The four treatment groups (uninfected females, infected
62 females, uninfected males, and infected males) were maintained in isolation until they were 3 to
63 5 days old. We then measured the locomotor activity of the batch of flies using a 16-chamber
64 flow-through respirometry and data acquisition system (MAVEN, Sable Systems International).
65 The MAVEN has 16 2.4 ml volume polycarbonate animal chambers and an activity board that
66 uses infrared light (invisible to flies) to monitor animal activity in each chamber, sampled at 1
67 Hz (Figure S1). Individual flies were aspirated into a randomly assigned chamber and allowed to
68 adjust to the new environment for 0.5 hours. Activity measurements were then recorded over a 3-
69 hour period between the hours of 0900 and 1600.

70 The raw outputs from the activity sensors were transformed into the activity index
71 absolute distance sums (ADS). We calculated ADS by first calculating the cumulative sum of the
72 absolute difference between consecutive activity readings, and then calculating the slope of
73 cumulative activity vs. time [65,66]. We used mean ADS over the 3-hour period as our estimate
74 of locomotor activity for each fly; however, our analyses were robust regardless of how we
75 quantified activity (see Supplemental Methods). We found that the mean ADS activity data
76 required a transformation for statistical analysis; however, a single data transformation was not
77 suitable for all host species. We used a log transformation of mean ADS for *D. simulans*, *D.*

78 *suzukii*, *D. auraria*, *D. mauritiana*, and *D. sechellia*, and a square root transformation for *D.*
79 *melanogaster*, *D. yakuba*, *D. santomea*, and *D. teissieri*. We present a full statistical analysis of
80 all datasets in Tables S2 and S3, respectively.

81 We used the log- and square root-transformed mean ADS data as dependent variables in
82 linear models. We included infection status, sex, and an infection-by-sex interaction effect as
83 independent variables, as well as additional independent variables to account for other potential
84 sources of activity variation: randomly assigned animal chamber (1-16), experimental start time,
85 mean water vapor (ppt), mean relative humidity (%), mean temperature (°C), and mean light
86 intensity (lux) [65,66]. We evaluated the significance of individual effects using *F* tests and type
87 III sum of squares using the “Anova” function in the *car* R package [67,68].

88

89 *Wolbachia* phylogenomic analysis

90 We used publicly available *Wolbachia* genome assemblies [20,42,43,59,69–71], and new
91 Illumina sequencing, to generate a Bayesian phylogram [20] (see Supplemental Methods).
92 *Wolbachia* effects on host activity were especially common for *w*Ri-like *Wolbachia*, so we used
93 the phylogram to test whether *Wolbachia* effects on hosts exhibit phylogenetic signal. First, we
94 treated *Wolbachia* effects on host locomotor activity as a binary trait and tested for phylogenetic
95 signal using the *D* statistic [72], implemented in the *caper* R package [73]. Second, we treated
96 *Wolbachia* effects on activity as a continuous trait and tested for phylogenetic signal using
97 Pagel’s lambda (λ) [74]. Here, we analyzed each sex separately, because we found significant
98 infection-by-sex interaction effects on activity (Tables S2 and S3). For each sex, we extracted the
99 least-square (LS) mean ADS for infected and uninfected flies from the linear models (Tables S2
100 and S3), and used the change in LS mean activity as a continuous character to calculate the
101 maximum likelihood value of Pagel’s λ [74,75]. We used a likelihood ratio test to compare our
102 fitted value of λ to a model assuming no phylogenetic signal ($\lambda = 0$) using the “phylosig”
103 function in the R package *phytools* [76].

104

105 RESULTS

106 *Wolbachia* infections modify host locomotor activity

107 We assayed the locomotor activity of 3,104 flies (Figure 1). *Wolbachia* had a significant
108 effect on the activity of six host genotypes, including hosts infected with both A- and B-group

109 *Wolbachia*. Interestingly, the direction of *Wolbachia* effects on host activity varied by genotype
110 and sex (Figure 2). We found a significant *Wolbachia* infection-by-sex interaction effect for the
111 *wMelCS-D. melanogaster* genotype that increased male activity ($F = 4.566$, $P = 0.033$; Table
112 S3). We also found a significant infection-by-sex effect for the *wRi-D. simulans* genotype, but
113 *Wolbachia* increased female activity ($F = 8.150$, $P = 0.005$; Table S2). The two other closely
114 related *wRi*-like *Wolbachia*, *wSuz* and *wAur*, also had significant effects on host activity. The
115 *wSuz-D. suzukii* genotype had a significant main effect of *Wolbachia* that reduced host activity
116 ($F = 11.311$, $P < 0.001$; Table S2), and the *wAur-D. auraria* genotype had a significant
117 infection-by-sex interaction that reduced female activity ($F = 6.584$, $P = 0.011$; Table S2). The
118 *wHa-D. simulans* genotype had a significant main effect of *Wolbachia* that increased host
119 activity ($F = 7.764$, $P = 0.006$; Table S2). Lastly, we found the *wHa-wNo* co-infected *D.*
120 *simulans* genotype had a significant infection-by-sex interaction effect that reduced male activity
121 ($F = 7.076$, $P = 0.008$; Table S2). Because this genotype is co-infected, we do not know the
122 relative contributions of *wHa* and *wNo* to variation in host activity. See the Supplemental Results
123 for a discussion of how other variables contributed to variation in locomotor activity.

124

125 *Limited evidence for phylogenetic signal*

126 We estimated a Bayesian phylogram of A- and B-group *Wolbachia* using 211 single-copy
127 genes of identical length in all *Wolbachia* genomes, spanning 178,569 bp (Figure 2). We then
128 tested whether closely related *Wolbachia* have similar effects on host activity. When treating
129 *Wolbachia* effects on activity as a binary trait, our estimate of $D = 0.322$ was low, but not
130 statistically different from a model of $D = 1$ assuming phylogenetic randomness ($P = 0.101$) or a
131 model of $D = 0$ with strong phylogenetic signal ($P = 0.198$). Simulations of similar phylogenies
132 with an increasing number of *Wolbachia* strains suggest that at least $N = 50$ strains are required
133 to differentiate our estimated value of $D = 0.322$ from a model of phylogenetic randomness ($D =$
134 1) (Figures S4 and S5). Thus, *Wolbachia* effects on host activity may exhibit phylogenetic
135 signal, but many more *Wolbachia* strains are required to test this hypothesis. Unfortunately, $N =$
136 50 strains are not presently available in culture. We also treated *Wolbachia* changes to host
137 activity as a continuous trait; however, we found that maximum likelihood fitted λ values were
138 extremely low, indicative of no phylogenetic signal. λ values generated from the LS mean log-
139 transformed ADS data were not statistically different from zero for females ($\lambda < 0.001$, $P = 1$) or

140 males ($\lambda < 0.001$, $P = 1$). This was also true when we repeated the analyses for the LS mean
141 square root-transformed ADS data for females ($\lambda < 0.001$, $P = 1$) and males ($\lambda < 0.001$, $P = 1$).

142

143 DISCUSSION

144 Our analyses suggest that *Wolbachia* commonly alter host locomotor activity, which may
145 affect host fitness. Locomotion is a basic host activity underlying many ecologically important
146 behaviors, including foraging, thermoregulation, and mate seeking. In combination with our
147 recent work demonstrating pervasive effects of A- and B-group *Wolbachia* on host temperature
148 preference [20], we posit that *Wolbachia* infections may often alter host behavior.

149 The *wRi*-like *Wolbachia* strains in our study (*wRi*, *wSuz*, and *wAur*) consistently altered
150 host activity. We found a low, but non-significant *D* value of 0.322, suggesting effects on host
151 activity may exhibit phylogenetic signal; although, an excessive number of *Wolbachia* strains are
152 required to test this hypothesis. Our findings are consistent with prior experiments demonstrating
153 that *wRi* increased female *D. simulans* activity in response to olfactory cues [77,78]. We
154 hypothesize that the prominence of *wRi*-like *Wolbachia* effects on host activity relative to other
155 strains may be due to variation in *Wolbachia* tissue localization [49,52]. *wRi* occurs at high titer
156 in adult *D. simulans* brains and localizes to specific regions, whereas *wMel* shows a relatively
157 even distribution in *D. melanogaster* [52]. *wRi* also occurs at higher titer in the ventral nerve
158 cord, which is a major neural circuit center for motor activities such as walking [52,79–81].
159 Future experiments should compare *Wolbachia* titer and localization in adult brains for *wRi*-like
160 variants and strains that do not alter locomotor activity.

161 We also found considerable variation in the direction and sex-bias of *Wolbachia* effects
162 on locomotor activity (Figure 2). *Wolbachia* decreased activity for *wSuz*, *wAur*, and the *wNo*-
163 *wHa* co-infection, whereas *wMelCS*, *wRi*, and *wHa* increased activity. These effects were
164 female-biased for *wRi* and *wAur*, but male-biased for *wMelCS* and *wNo-wHa*. This variation had
165 no relationship to the *Wolbachia* phylogeny, because we found no evidence for phylogenetic
166 signal when measuring *Wolbachia* effects on females and males as a continuous trait ($\lambda < 0.001$).
167 Specific *Wolbachia* effects on host activity may depend on interactions with the host
168 background. For example, our work and others' suggests that identical *wMelCS* variants have
169 different effects on *D. melanogaster* temperature preference depending on the host background
170 [20,57,58]. Host genomes also modify *Wolbachia* titer [82], *Wolbachia* maternal transmission

171 [83], components of host fitness [84–87], and the strength of cytoplasmic incompatibility [88–
172 90].

173 Changes to host activity could underlie *Wolbachia*-induced behaviors that promote
174 infection spread. For example, *wMel*-infected *D. melanogaster* have higher field recapture rates
175 than uninfected flies [91], and long distance dispersal of the spider *Erigone atra* is altered by
176 *Rickettsia*, an endosymbiont closely related to *Wolbachia* [92]. Our own work suggests
177 *Wolbachia* may alter host temperature preference to promote *Wolbachia* replication within host
178 bodies [20]. Other experiments suggest that *wMel* and *wRi* may influence male mating rate
179 [93,94]. Alternatively, hosts may be modifying their own behavior as a response to *Wolbachia*
180 infection. Several studies indicate that *wMel* alters circadian activity and sleep patterns of *D.*
181 *melanogaster* [52,54–56]. For example, Bi et al. [55] report that *wMel* increases sleep time,
182 which could represent a host immune response to infection [19]. Ultimately, these effects on host
183 behavior factor into how *Wolbachia* influence host fitness, which determines the spread and
184 persistence of *Wolbachia* in host populations [4,95–98]. Because locomotor activity is such a
185 fundamental host behavior, our results suggest *Wolbachia* may have complex and variable
186 effects on many components of host fitness.

187

188 **ACKNOWLEDGEMENTS**

189 We thank TBW for lab assistance and WRC for help with bioinformatic analyses. The
190 Cooper lab group and MT provided valuable feedback that improved the manuscript. This work
191 was supported by NIGMS of the NIH under award R35GM124701 to BSC.

192

193 **DATA AVAILABILITY**

194 All data and code are available on Dryad (doi:10.5061/dryad.6t1g1jwxv; temporary URL:
195 <https://datadryad.org/stash/share/D4Yi8zYFchXE-LIFgTc6S91s2Nl5Afcwu4-FkxJZPaA>).
196 Genome assemblies will be deposited on GenBank upon acceptance.

197 **FIGURE LEGENDS**

198 **Figure 1.** Activity of uninfected and infected flies for each sex of each genotype. Activity is
199 measured as mean absolute distance sums (ADS). Significance was evaluated using linear
200 models (Tables S2 and S3).

201

202 **Figure 2. (A)** Estimated Bayesian phylogram for A- and B-group *Wolbachia* strains. The
203 divergence estimate for A- and B-groups is superimposed from Meany et al. [59]. All nodes have
204 Bayesian posterior probabilities of 1. **(B)** *Wolbachia* effects on host activity scored as a binary
205 trait: *Wolbachia* significantly altered host activity (black circle) or had no effect (white circle).
206 **(C)** *Wolbachia* effects on activity scored as a continuous trait: the change in least-square (LS)
207 mean log-transformed activity (ADS) for each sex. LS means were generated from linear models
208 (Table S2). LS mean square root-transformed ADS data are shown in Figure S3.

209 **REFERENCES**

- 210 1. Moran NA, McCutcheon JP, Nakabachi A. 2008 Genomics and evolution of heritable
211 bacterial symbionts. *Annual Review of Genetics* **42**, 165–190.
- 212 2. McFall-Ngai M *et al.* 2013 Animals in a bacterial world, a new imperative for the life
213 sciences. *Proceedings of the National Academy of Sciences* **110**, 3229–3236.
- 214 3. McCutcheon JP, Boyd BM, Dale C. 2019 The life of an insect endosymbiont from the
215 cradle to the grave. *Current Biology* **29**, R485–R495.
- 216 4. Hoffmann AA, Turelli M. 1997 Cytoplasmic incompatibility in insects. In *Influential*
217 *passengers: inherited microorganisms and arthropod reproduction* (eds SL O’Neill, AA
218 Hoffmann, JH Werren), pp. 42–80. Oxford University Press.
- 219 5. Werren JH, Baldo L, Clark ME. 2008 *Wolbachia*: master manipulators of invertebrate
220 biology. *Nature Reviews Microbiology* **6**, 741–751.
- 221 6. Hurst G, Hurst L, Majerus M. 1997 Cytoplasmic sex-ratio distorters. In *Influential*
222 *passengers: inherited microorganisms and arthropod reproduction* (eds SL O’Neill, AA
223 Hoffmann, JH Werren), pp. 125–154. Oxford University Press.
- 224 7. Shropshire JD, Leigh B, Bordenstein SR. 2020 Symbiont-mediated cytoplasmic
225 incompatibility: what have we learned in 50 years? *Elife* **9**, e61989.
- 226 8. Baumann P. 2005 Biology of bacteriocyte-associated endosymbionts of plant sap-sucking
227 insects. *Annual Review of Microbiology* **59**, 155–189.
- 228 9. Douglas AE. 2009 The microbial dimension in insect nutritional ecology. *Functional*
229 *Ecology* **23**, 38–47.
- 230 10. Brumin M, Kontsedalov S, Ghanim M. 2011 *Rickettsia* influences thermotolerance in the
231 whitefly *Bemisia tabaci* B biotype. *Insect Science* **18**, 57–66.
- 232 11. Mueller UG *et al.* 2011 Evolution of cold-tolerant fungal symbionts permits winter
233 fungiculture by leafcutter ants at the northern frontier of a tropical ant–fungus symbiosis.
234 *Proceedings of the National Academy of Sciences* **108**, 4053–4056.
- 235 12. Hedges LM, Brownlie JC, O’Neill SL, Johnson KN. 2008 *Wolbachia* and virus protection
236 in insects. *Science* **322**, 702–702.
- 237 13. Teixeira L, Ferreira Á, Ashburner M. 2008 The bacterial symbiont *Wolbachia* induces
238 resistance to RNA viral infections in *Drosophila melanogaster*. *PLoS Biology* **6**, e1000002.
- 239 14. Jaenike J, Unckless R, Cockburn SN, Boelio LM, Perlman SJ. 2010 Adaptation via
240 symbiosis: recent spread of a *Drosophila* defensive symbiont. *Science* **329**, 212–215.

- 241 15. Oliver KM, Smith AH, Russell JA. 2014 Defensive symbiosis in the real world—advancing
242 ecological studies of heritable, protective bacteria in aphids and beyond. *Functional*
243 *Ecology* **28**, 341–355.
- 244 16. Feldhaar H. 2011 Bacterial symbionts as mediators of ecologically important traits of insect
245 hosts. *Ecological Entomology* **36**, 533–543.
- 246 17. Goodacre SL, Martin OY. 2012 Modification of insect and arachnid behaviours by
247 vertically transmitted endosymbionts: infections as drivers of behavioural change and
248 evolutionary novelty. *Insects* **3**, 246–261.
- 249 18. Schretter CE, Vielmetter J, Bartos I, Marka Z, Marka S, Argade S, Mazmanian SK. 2018 A
250 gut microbial factor modulates locomotor behaviour in *Drosophila*. *Nature* **563**, 402–406.
- 251 19. Bi J, Wang Y-F. 2020 The effect of the endosymbiont *Wolbachia* on the behavior of insect
252 hosts. *Insect Science* **27**, 846–858.
- 253 20. Hague MT, Caldwell CN, Cooper BS. 2020 Pervasive effects of *Wolbachia* on host
254 temperature preference. *mBio* **11**.
- 255 21. Hosokawa T, Fukatsu T. 2020 Relevance of microbial symbiosis to insect behavior.
256 *Current Opinion in Insect Science* **39**, 91–100.
- 257 22. Thomas F, Adamo S, Moore J. 2005 Parasitic manipulation: where are we and where
258 should we go? *Behavioural Processes* **68**, 185–199.
- 259 23. Lefevre T, Thomas F. 2008 Behind the scene, something else is pulling the strings:
260 emphasizing parasitic manipulation in vector-borne diseases. *Infection, Genetics and*
261 *Evolution* **8**, 504–519.
- 262 24. Poulin R. 2010 Parasite manipulation of host behavior: an update and frequently asked
263 questions. In *Advances in the Study of Behavior*, pp. 151–186. Elsevier.
- 264 25. van Houte S, Ros VI, van Oers MM. 2013 Walking with insects: molecular mechanisms
265 behind parasitic manipulation of host behaviour. *Molecular Ecology* **22**, 3458–3475.
- 266 26. Heil M. 2016 Host manipulation by parasites: cases, patterns, and remaining doubts.
267 *Frontiers in Ecology and Evolution* **4**, 80.
- 268 27. Vale PF, Siva-Jothy A, Morrill A, Forbes MR. 2018 The influence of parasites. In *Insect*
269 *behavior: from mechanisms to ecological and evolutionary consequences*, pp. 273–291.
270 Oxford University Press Oxford.
- 271 28. Weinersmith KL. 2019 What’s gotten into you?: A review of recent research on parasitoid
272 manipulation of host behavior. *Current Opinion in Insect Science*

- 273 29. Louis C, Jourdan M, Cabanac M. 1986 Behavioral fever and therapy in a rickettsia-infected
274 Orthoptera. *American Journal of Physiology-Regulatory, Integrative and Comparative*
275 *Physiology* **250**, R991–R995.
- 276 30. Fedorka KM, Kutch IC, Collins L, Musto E. 2016 Cold temperature preference in
277 bacterially infected *Drosophila melanogaster* improves survival but is remarkably
278 suboptimal. *Journal of Insect Physiology* **93**, 36–41.
- 279 31. Adelman JS, Martin LB. 2009 Vertebrate sickness behaviors: adaptive and integrated
280 neuroendocrine immune responses. *Integrative and Comparative Biology* **49**, 202–214.
- 281 32. Opp MR. 2009 Sleeping to fuel the immune system: mammalian sleep and resistance to
282 parasites. *BMC Evolutionary Biology* **9**, 1–3.
- 283 33. Kuo T-H, Pike DH, Beizaeipour Z, Williams JA. 2010 Sleep triggered by an immune
284 response in *Drosophila* is regulated by the circadian clock and requires the NFκB Relish.
285 *BMC Neuroscience* **11**, 1–12.
- 286 34. Kuo T-H, Williams JA. 2014 Increased sleep promotes survival during a bacterial infection
287 in *Drosophila*. *Sleep* **37**, 1077–1086.
- 288 35. Zug R, Hammerstein P. 2012 Still a host of hosts for *Wolbachia*: Analysis of recent data
289 suggests that 40% of terrestrial arthropod species are infected. *PLoS One* **7**, e38544.
- 290 36. Weinert LA, Araujo-Jnr EV, Ahmed MZ, Welch JJ. 2015 The incidence of bacterial
291 endosymbionts in terrestrial arthropods. *Proceedings of the Royal Society B: Biological*
292 *Sciences* **282**, 20150249.
- 293 37. Bandi C, Anderson TJ, Genchi C, Blaxter ML. 1998 Phylogeny of *Wolbachia* in filarial
294 nematodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **265**,
295 2407–2413.
- 296 38. O’Neill SL, Giordano R, Colbert AM, Karr TL, Robertson HM. 1992 16S rRNA
297 phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic
298 incompatibility in insects. *Proceedings of the National Academy of Sciences* **89**, 2699–
299 2702.
- 300 39. Raychoudhury R, Baldo L, Oliveira DC, Werren JH. 2009 Modes of acquisition of
301 *Wolbachia*: Horizontal transfer, hybrid introgression, and codivergence in the *Nasonia*
302 species complex. *Evolution* **63**, 165–183.
- 303 40. Conner WR, Blaxter ML, Anfora G, Ometto L, Rota-Stabelli O, Turelli M. 2017 Genome
304 comparisons indicate recent transfer of wRi-like *Wolbachia* between sister species
305 *Drosophila suzukii* and *D. subpulchrella*. *Ecology and Evolution* **7**, 9391–9404.
- 306 41. Gerth M, Bleidorn C. 2017 Comparative genomics provides a timeframe for *Wolbachia*
307 evolution and exposes a recent biotin synthesis operon transfer. *Nature Microbiology* **2**,
308 16241.

- 309 42. Turelli M *et al.* 2018 Rapid global spread of *w*Ri-like *Wolbachia* across multiple
310 *Drosophila*. *Current Biology* **28**, 963–971.e8.
- 311 43. Cooper BS, Vanderpool D, Conner WR, Matute DR, Turelli M. 2019 *Wolbachia*
312 acquisition by *Drosophila yakuba*-clade hosts and transfer of incompatibility loci between
313 distantly related *Wolbachia*. *Genetics* **212**, 1399–1419.
- 314 44. Prout T. 1994 Some evolutionary possibilities for a microbe that causes incompatibility in
315 its host. *Evolution* **48**, 909–911.
- 316 45. Turelli M. 1994 Evolution of incompatibility-inducing microbes and their hosts. *Evolution*
317 **48**, 1500–1513.
- 318 46. Weeks AR, Turelli M, Harcombe WR, Reynolds KT, Hoffmann AA. 2007 From parasite to
319 mutualist: Rapid evolution of *Wolbachia* in natural populations of *Drosophila*. *PLoS*
320 *Biology* **5**, e114.
- 321 47. Haygood R, Turelli M. 2009 Evolution of incompatibility-inducing microbes in subdivided
322 host populations. *Evolution* **63**, 432–447.
- 323 48. Dobson SL, Bourtzis K, Braig HR, Jones BF, Zhou W, Rousset F, O’Neill SL. 1999
324 *Wolbachia* infections are distributed throughout insect somatic and germ line tissues. *Insect*
325 *Biochemistry and Molecular Biology* **29**, 153–160.
- 326 49. Albertson R, Casper-Lindley C, Cao J, Tram U, Sullivan W. 2009 Symmetric and
327 asymmetric mitotic segregation patterns influence *Wolbachia* distribution in host somatic
328 tissue. *Journal of Cell Science* **122**, 4570–4583.
- 329 50. Casper-Lindley C, Kimura S, Saxton DS, Essaw Y, Simpson I, Tan V, Sullivan W. 2011
330 Rapid fluorescence-based screening for *Wolbachia* endosymbionts in *Drosophila* germ line
331 and somatic tissues. *Applied Environmental Microbiology* **77**, 4788–4794.
- 332 51. Pietri JE, DeBruhl H, Sullivan W. 2016 The rich somatic life of *Wolbachia*.
333 *MicrobiologyOpen* **5**, 923–936.
- 334 52. Albertson R, Tan V, Leads RR, Reyes M, Sullivan W, Casper-Lindley C. 2013 Mapping
335 *Wolbachia* distributions in the adult *Drosophila* brain. *Cellular Microbiology* **15**, 1527–
336 1544.
- 337 53. Fleury F, Vavre F, Ris N, Fouillet P, Bouletreau M. 2000 Physiological cost induced by the
338 maternally-transmitted endosymbiont *Wolbachia* in the *Drosophila* parasitoid *Leptopilina*
339 *heterotoma*. *Parasitology* **121**, 493–500.
- 340 54. Vale PF, Jardine MD. 2015 Sex-specific behavioural symptoms of viral gut infection and
341 *Wolbachia* in *Drosophila melanogaster*. *Journal of Insect Physiology* **82**, 28–32.
- 342 55. Bi J, Sehgal A, Williams JA, Wang Y-F. 2018 *Wolbachia* affects sleep behavior in
343 *Drosophila melanogaster*. *Journal of Insect Physiology* **107**, 81–88.

- 344 56. Morioka E, Oida M, Tsuchida T, Ikeda M. 2018 Nighttime activities and peripheral clock
345 oscillations depend on *Wolbachia* endosymbionts in flies. *Scientific reports* **8**, 15432.
- 346 57. Arnold PA, Levin SC, Stevanovic AL, Johnson KN. 2019 *Drosophila melanogaster*
347 infected with *Wolbachia* strain wMelCS prefer cooler temperatures. *Ecological Entomology*
348 **44**, 287–290.
- 349 58. Truitt AM, Kapun M, Kaur R, Miller WJ. 2019 *Wolbachia* modifies thermal preference in
350 *Drosophila melanogaster*. *Environmental Microbiology* **21**, 3259–3268.
- 351 59. Meany MK, Conner WR, Richter SV, Bailey JA, Turelli M, Cooper BS. 2019 Loss of
352 cytoplasmic incompatibility and minimal fecundity effects explain relatively low
353 *Wolbachia* frequencies in *Drosophila mauritiana*. *Evolution* **73**, 1278–1295.
- 354 60. O’Neill SL, Karr TL. 1990 Bidirectional incompatibility between conspecific populations
355 of *Drosophila simulans*. *Nature* **348**, 178–180.
- 356 61. Mercot H, Llorente B, Jacques M, Atlan A, Montchamp-Moreau C. 1995 Variability within
357 the Seychelles cytoplasmic incompatibility system in *Drosophila simulans*. *Genetics* **141**,
358 1015–1023.
- 359 62. Rousset F, Solignac M. 1995 Evolution of single and double *Wolbachia* symbioses during
360 speciation in the *Drosophila simulans* complex. *Proceedings of the National Academy of*
361 *Sciences* **92**, 6389–6393.
- 362 63. James A, Dean M, McMahon M, Ballard J. 2002 Dynamics of double and single *Wolbachia*
363 infections in *Drosophila simulans* from New Caledonia. *Heredity* **88**, 182–189.
- 364 64. Ballard J, Melvin R. 2007 Tetracycline treatment influences mitochondrial metabolism and
365 mtDNA density two generations after treatment in *Drosophila*. *Insect Molecular Biology*
366 **16**, 799–802.
- 367 65. Videlier M, Rundle HD, Careau V. 2019 Sex-specific among-individual covariation in
368 locomotor activity and resting metabolic rate in *Drosophila melanogaster*. *The American*
369 *Naturalist* **194**, E164–E176.
- 370 66. Videlier M, Careau V, Wilson AJ, Rundle HD. 2021 Quantifying selection on standard
371 metabolic rate and body mass in *Drosophila melanogaster*. *Evolution* **75**, 130–140.
- 372 67. Fox J, Weisberg S. 2019 *An R Companion to Applied Regression*. Third. Thousand Oaks
373 CA: Sage. See <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>.
- 374 68. R Core Team. 2018 *R: A language and environment for statistical computing*. Vienna,
375 Austria: R Foundation for Statistical Computing. See <https://www.R-project.org/>.
- 376 69. Klasson L *et al.* 2009 The mosaic genome structure of the *Wolbachia* wRi strain infecting
377 *Drosophila simulans*. *Proceedings of the National Academy of Sciences* **106**, 5725–5730.

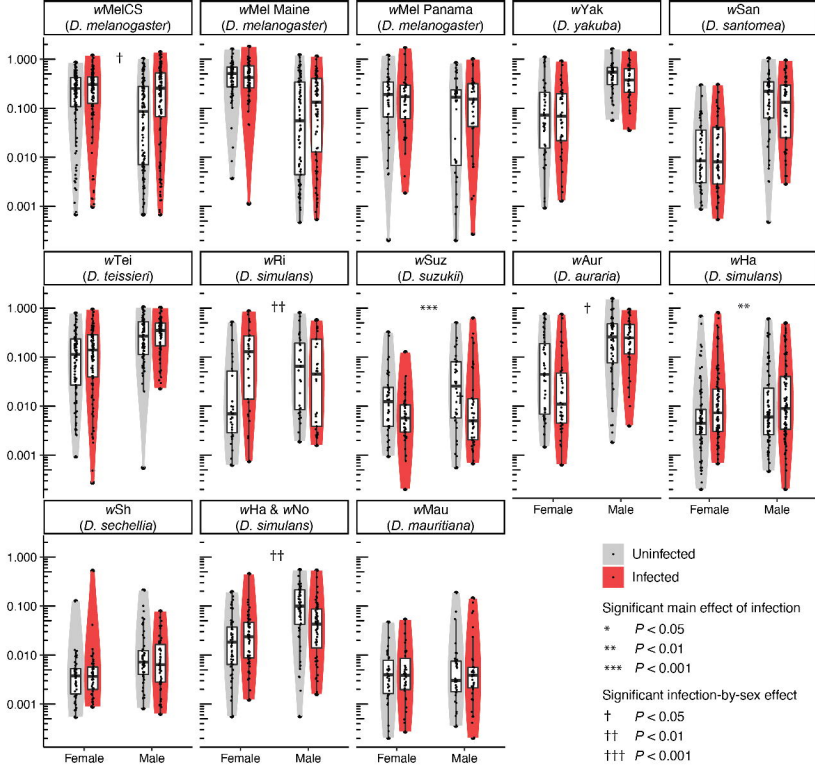
- 378 70. Ellegaard KM, Klasson L, Näslund K, Bourtzis K, Andersson SG. 2013 Comparative
379 genomics of *Wolbachia* and the bacterial species concept. *PLoS Genetics* **9**.
- 380 71. Siozios S, Cestaro A, Kaur R, Pertot I, Rota-Stabelli O, Anfora G. 2013 Draft genome
381 sequence of the *Wolbachia* endosymbiont of *Drosophila suzukii*. *Genome announcements*
382 **1**, e00032-13.
- 383 72. Fritz SA, Purvis A. 2010 Selectivity in mammalian extinction risk and threat types: a new
384 measure of phylogenetic signal strength in binary traits. *Conservation Biology* **24**, 1042–
385 1051.
- 386 73. Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. 2018 *caper*:
387 *Comparative Analyses of Phylogenetics and Evolution in R*. See [https://CRAN.R-](https://CRAN.R-project.org/package=caper)
388 [project.org/package=caper](https://CRAN.R-project.org/package=caper).
- 389 74. Pagel M. 1999 Inferring the historical patterns of biological evolution. *Nature* **401**, 877–
390 884.
- 391 75. Freckleton RP, Harvey PH, Pagel M. 2002 Phylogenetic analysis and comparative data: a
392 test and review of evidence. *The American Naturalist* **160**, 712–726.
- 393 76. Revell LJ. 2012 phytools: an R package for phylogenetic comparative biology (and other
394 things). *Methods in Ecology and Evolution* **3**, 217–223.
- 395 77. Peng Y, Nielsen JE, Cunningham JP, McGraw EA. 2008 *Wolbachia* infection alters
396 olfactory-cued locomotion in *Drosophila* spp. *Applied Environmental Microbiology* **74**,
397 3943–3948.
- 398 78. Peng Y, Wang Y. 2009 Infection of *Wolbachia* may improve the olfactory response of
399 *Drosophila*. *Chinese Science Bulletin* **54**, 1369–1375.
- 400 79. Burrows M, Laurent G, Field L. 1988 Proprioceptive inputs to nonspiking local
401 interneurons contribute to local reflexes of a locust hindleg. *Journal of Neuroscience* **8**,
402 3085–3093.
- 403 80. Laurent G, Burrows M. 1988 A population of ascending intersegmental interneurons in the
404 locust with mechanosensory inputs from a hind leg. *Journal of Comparative Neurology*
405 **275**, 1–12.
- 406 81. Yellman C, Tao H, He B, Hirsh J. 1997 Conserved and sexually dimorphic behavioral
407 responses to biogenic amines in decapitated *Drosophila*. *Proceedings of the national*
408 *academy of sciences* **94**, 4131–4136.
- 409 82. Funkhouser-Jones LJ, van Opstal EJ, Sharma A, Bordenstein SR. 2018 The maternal effect
410 gene *Wds* controls *Wolbachia* titer in *Nasonia*. *Current Biology* **28**, 1692–1702.
- 411 83. Serbus LR, Sullivan W. 2007 A cellular basis for *Wolbachia* recruitment to the host
412 germline. *PLoS Pathogens* **3**, e190.

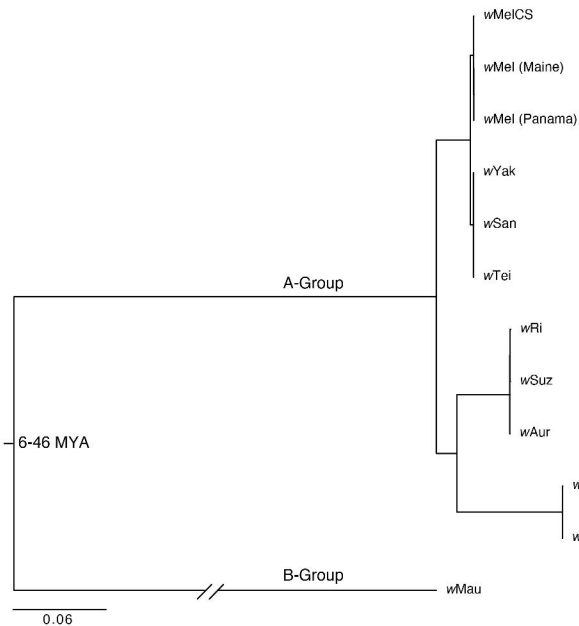
- 413 84. Fry A, Palmer M, Rand D. 2004 Variable fitness effects of *Wolbachia* infection in
414 *Drosophila melanogaster*. *Heredity* **93**, 379.
- 415 85. Dean MD. 2006 A *Wolbachia*-associated fitness benefit depends on genetic background in
416 *Drosophila simulans*. *Proceedings of the Royal Society of London B: Biological Sciences*
417 **273**, 1415–1420.
- 418 86. Gruntenko NE, Karpova EK, Adonyeva NV, Andreenkova OV, Burdina EV, Ilinsky YY,
419 Bykov RA, Menshanov PN, Rauschenbach IY. 2019 *Drosophila* female fertility and
420 juvenile hormone metabolism depends on the type of *Wolbachia* infection. *Journal of*
421 *Experimental Biology* **222**, jeb195347.
- 422 87. Serga SV, Maistrenko OM, Matiysiv NP, Vaiserman AM, Kozeretska IA. 2021 Effects of
423 *Wolbachia* infection on fitness-related traits in *Drosophila melanogaster*. *Symbiosis*
424 (doi:10.1007/s13199-020-00743-3)
- 425 88. Reynolds KT, Hoffmann AA. 2002 Male age, host effects and the weak expression or non-
426 expression of cytoplasmic incompatibility in *Drosophila* strains infected by maternally
427 transmitted *Wolbachia*. *Genetics Research* **80**, 79–87.
- 428 89. Cooper BS, Ginsberg PS, Turelli M, Matute DR. 2017 *Wolbachia* in the *Drosophila yakuba*
429 complex: Pervasive frequency variation and weak cytoplasmic incompatibility, but no
430 apparent effect on reproductive isolation. *Genetics* **205**, 333–351.
- 431 90. Cattel J *et al.* 2018 Back and forth *Wolbachia* transfers reveal efficient strains to control
432 spotted wing *Drosophila* populations. *Journal of Applied Ecology* **55**, 2408–2418.
- 433 91. Caragata EP, Real KM, Zalucki MP, McGraw EA. 2011 *Wolbachia* infection increases
434 recapture rate of field-released *Drosophila melanogaster*. *Symbiosis* **54**, 55.
- 435 92. Goodacre SL, Martin OY, Bonte D, Hutchings L, Woolley C, Ibrahim K, Thomas CG,
436 Hewitt GM. 2009 Microbial modification of host long-distance dispersal capacity. *BMC*
437 *Biology* **7**, 1–8.
- 438 93. De Crespigny FC, Pitt T, Wedell N. 2006 Increased male mating rate in *Drosophila* is
439 associated with *Wolbachia* infection. *Journal of Evolutionary Biology* **19**, 1964–1972.
- 440 94. De Crespigny FEC, Wedell N. 2007 Mate preferences in *Drosophila* infected with
441 *Wolbachia*? *Behavioral Ecology and Sociobiology* **61**, 1229.
- 442 95. Hoffmann AA, Turelli M, Harshman LG. 1990 Factors affecting the distribution of
443 cytoplasmic incompatibility in *Drosophila simulans*. *Genetics* **126**, 933–948.
- 444 96. Kriesner P, Hoffmann AA. 2018 Rapid spread of a *Wolbachia* infection that does not affect
445 host reproduction in *Drosophila simulans* cage populations. *Evolution* **72**, 1475–1487.
- 446 97. Ross PA, Turelli M, Hoffmann AA. 2019 Evolutionary ecology of *Wolbachia* releases for
447 disease control. *Annual Review of Genetics* **53**.

- 448 98. Kriesner P, Conner WR, Weeks AR, Turelli M, Hoffmann AA. 2016 Persistence of a
449 *Wolbachia* infection frequency cline in *Drosophila melanogaster* and the possible role of
450 reproductive dormancy. *Evolution* **70**, 979–997.

451

Mean activity (ADS) over 3 hours



A**B**